Association between -174G/C and -572G/C interleukin 6 gene polymorphisms and severe radiographic damage to the hands of Mexican patients with rheumatoid arthritis: a preliminary report

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ABSTRACT. Several interleukin 6 gene (*IL6*) polymorphisms are implicated in susceptibility to rheumatoid arthritis (RA). It has not yet been established with certainty if these polymorphisms are associated with the severe radiographic damage observed in some RA patients, particularly those with the development of joint bone ankylosis (JBA). The objective of the present study was to evaluate the association between severe radiographic damage in hands and the -174G/C and -572G/C *IL6* polymorphisms in Mexican Mestizo people with RA. Mestizo adults with RA and long disease duration (>5 years) were classified into two groups according to the radiographic damage in their hands: a) severe radiographic damage (JBA and/or joint bone subluxations) and b) mild or moderate radiographic damage. We compared the differences in genotype and allele frequencies of -174G/C and -572G/C *IL6* polymorphisms (genotyped using polymerase chain reaction-restriction fragment length polymorphism) between these two groups. Our findings indicated that the -174G/C polymorphism of *IL6* is associated with severe joint radiographic damage [maximum likelihood odds ratios (MLE_OR): 8.03; 95%CI 1.22-187.06; P = 0.03], whereas the -572G/C polymorphism of *IL6* exhibited no such association (MLE_OR: 1.5; 95%CI 0.52-4.5; P = 0.44). Higher anti-cyclic citrullinated peptide antibody levels were associated with more severe joint radiographic damage (P = 0.04). We

conclude that there is a relevant association between the -174G/C *IL6* polymorphism and severe radiographic damage. Future studies in other populations are required to confirm our findings.

Key words: Rheumatoid arthritis; Gene; Interleukin 6; Hands; Steinbrocker radiographic classification

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease characterized by a chronic inflammation of the synovial joints, synovial hyperplasia, and pannus, and, in the long term, structural damage with cartilage and bone destruction (McInnes and Schett, 2011). RA leads to progressive disability in many patients and constitutes a high economic burden for public health systems (Firestein, 2003). It has been reported that joint erosions develop in 96% of RA patients within 10 years of the initial diagnosis (Lindqvist et al., 2003). The worst outcome of joint damage is manifested through subluxations and joint bone ankylosis (JBA). These subluxations and JBA can occur in many parts of the body, including the hands, feet, hips, and knees. However, JBA is more frequently observed in the hands, whereby a patient's ability to perform everyday activities is compromised (Machold et al., 2002). JBA in the hands has been reported in approximately 23% of patients with RA. Individuals with ankylosed joints in the hands have limited mobility, which diminishes their quality of life (Kaye et al., 1987).

Many authors have reported that the severity of radiographic structural damage is closely related to long-term disability and loss of function (Machold et al., 2002; Scott, 2003). Although different scales have been used for the assessment of radiographic and structural damage in RA (Sharp et al., 1971; Larsen, 1973; Lindqvist et al., 2005), the Steinbrocker radiographic classification is one of the best-known and simplest scales applied in clinical contexts. The Steinbrocker radiographic classification takes into account global radiographic damage in the hands and wrists, and is measured on a four-point scale, with JBA representing the maximum possible damage (Steinbrocker et al., 1949). Although the pathogenesis of JBA is multifactorial, certain cytokines are particularly implicated in the development of joint destruction. Bone ankylosis in RA is related to high levels of interleukin 6 (IL-6). According to Knudsen et al. (2008), IL-6 is closely related to radiographic progression in RA (Knudsen et al., 2008). IL-6 plays an important role in osteoclastogenesis, leading to cartilage and bone damage (Welsing et al., 2001; Mima and Nishimoto, 2009; Moots et al., 2009; Dayer and Choy, 2010). Published evidence indicates that the -174G/C (rs1800795) and -572G/C (rs1800796), single nucleotide polymorphisms of the interleukin 6 gene (*IL6*) promoter region, exert an influence on the rate of transcription of IL-6 protein, and may influence serum levels of IL-6 in patients with RA (Wang et al., 2013). Therefore, an evaluation of the role of these two *IL6* gene polymorphisms in the development of radiographic damage in the hands of RA patients is required.

Nevertheless, studies with the aim of investigate the association between radiographic joint destruction in RA and genetic variants in the *IL6* gene are limited, and their results vary according to ethnicity and race (Pawlik et al., 2005; Marinou et al., 2007; Ceccarelli et al., 2011; Pavkova Goldbergova et al., 2014). Some races experience more aggressive RA. RA and juvenile chronic arthritis appear to be more aggressive in Hispanics, who demonstrate more severe structural damage and higher disability rates compared with patients of other ethnic origins (Contreras-Yáñez et al., 2011; Pelajo et al., 2013). Therefore, a structured evaluation of the effect of *IL6*

gene polymorphisms on phenotype variants of severe RA is relevant for Hispanics. To the best of our knowledge, when we conducted the present research, there were no studies evaluating the relationship between *IL6* polymorphisms and JBA in Mexican patients with RA. Therefore, the aim of this study was to evaluate the association between severe radiographic damage in the hands of adult patients with RA and the -174G/C and -572G/C interleukin *IL6* gene polymorphisms.

MATERIAL AND METHODS

Design

The current sudy comprised a case-control study.

Clinical setting

This study included 61 unrelated adult patients with RA, and was performed at an outpatient rheumatology clinic of a secondary care hospital in Guadalajara, Mexico (Hospital General Regional 110, at Instituto Mexicano del Seguro Social. The inclusion criteria were: 1) the patients must have met the revised 1987 American College of Rheumatology criteria (Arnett et al., 1988); 2) the patients must have been Mexican Mestizo in origin (defined as individuals who, for three generations including their own, had been born in Mexico and who were descendants of the original autochthonous inhabitants of the region and of individuals who were mainly Spaniards) (Corona-Sanchez et al., 2012); and 3) the disease duration of the patients must have been ≥5 years from the onset of symptoms (we chose this cut-off point because disease duration is a confounder for severity of joint destruction and we consider that, to identify patients who may have developed severe joint bone damage, a minimum of 5 years is adequate). Only one RA patient per family was included in the study. We excluded patients who did not know their ancestral origin, patients who had an overlapping syndrome, patients who had incomplete information in the clinical chart about disease duration or other relevant variables, and patients with antecedents of poliomyelitis, amyotrophic lateral sclerosis, multiple sclerosis, or stroke. Patients with blood transfusions, with acute infection or diabetes mellitus were also excluded.

Clinical and laboratory assessments

A structured interview and a physical examination of all patients included in the study were conducted by two researchers to determine the sociodemographic and disease characteristics, as well as the patients' current therapy. Disease activity was assessed at the time of the study by a trained researcher using the disease activity score of 28 joints (DAS-28) (Shammas et al., 2010), whereas disability was evaluated using the Health Assessment Questionnaire-Disability index (Cardiel et al., 1993). Serum C-reactive protein (CRP) levels and rheumatoid factor (RF) levels were quantified in the serum by nephelometry, whereas anti-cyclic citrullinated peptide antibody (anti-CCP) levels and anti-mutated citrullinated vimentin antibody (anti-MCV) levels were quantified in the serum by enzyme-linked immunosorbent assay (ELISA), both using commercial kits (anti-CCP: Euroimmun, Medizinische Labordiagnostika, Germany; and anti-MCV: Orgentec Diagnostika GmbH, Mainz, Germany). The cut-off points for positivity were based on the manufacturer recommendations: a) positive CRP > 10 mg/L; b) positive RF > 20 IU/mL; and c) positive anti-CCP > 5 RU/mL and positive

anti-MCV > 20 U/mL. The cut-off points for anti-CCPs were validated in a previous study performed in our laboratory (Díaz-Toscano et al., 2014).

Radiographic assessment

At the time of evaluation, comparative anteroposterior and oblique hand and wrist radiographic images were obtained. These were examined by two trained rheumatologists blinded to the patients' clinical and laboratory characteristics. The radiographic images were scored using the Steinbrocker classification (Steinbrocker et al., 1949). The Steinbrocker radiographic stages in hands include: stage I (periarticular osteoporosis without evidence of destructive changes); stage II (periarticular osteoporosis and intraarticular erosions); stage III (periarticular osteoporosis, intraarticular erosions, and subluxations); and stage IV (presence of ankylosis).

For this study, we classified structural radiographic damage in hands into the following two groups: Group 1, which included patients with mild or moderate radiographic damage (Steinbrocker stages I or II), and Group 2, comprising patients with severe radiographic damage (Steinbrocker stages III or IV).

DNA extraction and *IL6* -174G/C and -572G/C genotyping

Genomic DNA was obtained by the Miller method (Miller et al., 1988) from the patients' peripheral blood collected in tubes containing ethylenediaminetetraacetic acid. The genotyping of the -174G/C and -572G/C polymorphisms was performed by trained personnel blinded to the patients' clinical characteristics. The genotypes were screened by an approach based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and *Sfa*NI and *Bsr*BI restriction endonucleases were used for the -174G/C and -572G/C polymorphisms, respectively, as described elsewhere (Zavaleta-Muñiz et al., 2013). The resulting fragments were analyzed by electrophoresis on a 6% polyacrylamide gel stained with silver nitrate. The resulting genotypes for both polymorphisms were classified into one of the following three categories: non-excisable homozygote (CC), excisable homozygote (GG), and heterozygote (CG).

Statistical analysis

Qualitative variables are expressed as numbers, and percentages and quantitative variables are presented as medians and ranges. The allele frequencies of both polymorphisms were determined by counting the observed genotype frequencies. The chi-square (χ^2) test was utilized for comparison between qualitative variables, and the Mann-Whitney U-test was used for comparison between quantitative variables. Clinical and serological quantitative characteristics were compared between different -174G/C or -572G/C genotypes using the Mann-Whitney U-test. We the computed maximum likelihood odds ratios (MLE_OR) and their 95% confidence intervals (95%CIs) to identify the risks associated with the different genotypes or alleles evaluated for the presence of severe joint radiographic damage in the hands (Steinbrocker stages III or IV). The significance value was set at $P \leq 0.05$. One-tailed P values for MLE_OR were computed and the Mid-P exact is shown. All statistical analyses were performed using the SPSS version 21.0 software (IBM Corporation, Armonk, NY, USA) and EPI INFO version 7.1 software (Epi Info™, Atlanta, GA, USA).

Ethics

Written voluntary consent to participate in molecular genetics analyses was obtained from all patients, and the study protocol was approved by a research committee from the participant center (approval No.: R-2009-1301-78). All study participants voluntarily provided written informed consent. All procedures in the protocol were performed in accordance with the Declaration of Helsinki guidelines.

RESULTS

We included 61 RA patients who had had the disease for at least 5 years. The patients' general characteristics are presented in Table 1.

Table 1. Clinical characteristics and genotype frequencies in patients with rheumatoid arthritis (RA).

Characteristics	RA (N = 61)
Females, N (%)	59 (96.7)
Age, years, median (range)	54 (34-79)
Disease duration, years, median (range)	12 (6-33)
DAS-28, median (range)	4 (2-7.4)
HAQ-Di, median (range)	0.6 (0-2.8)
CRP levels, mg/L, median (range)	12.9 (0-174)
CRP (+), N (%)	28 (45.9)
RF levels, IU/mL, median, (range)	29.40 (0-1566)
RF (+), N (%)	24 (39.3)
Anti-CCP levels, RU/mL, median (range)	27.6 (0.01-268)
Anti-CCP (+), N (%)	26 (42.6)
Anti-MCV levels, U/mL, median (range)	84.4 (1-228.5)
Anti-MCV (+), N (%)	34 (55.7)
Mild/moderate radiological damage (Steinbrocker stages I-II), N (%)	22 (36.1)
Severe radiological damage (Steinbrocker stages III-IV), N (%)	39 (63.9)
Genotype frequencies -174G/C of *IL6*, N (%)	
G/G	49 (80.3)
G/C	12 (19.7)
C/C	0 (0)
Genotype frequencies -572G/C of *IL6*, N (%)	
G/G	32 (52.5)
G/C	26 (42.6)
C/C	3 (4.9)
Medication	N (%)
Methotrexate	47 (77.0)
Chloroquine	13 (21.3)
Sulfasalazine	12 (19.70)
Leflunomide	19 (31.1)
Prednisone	52 (85.2)
Biologics*	7 (11.5)

*Biologics used: rituximab or etanercept. RA: rheumatoid arthritis; DAS-28: disease activity score of 28 joints, HAQ-DI: health assessment questionnaire-disability index; CRP: C-reactive protein; CRP (+) was considered when >10 mg/L; RF: rheumatoid factor; RF (+) was considered when >12 IU/mL (IU: international units); anti-CCP: anti-cyclic citrullinated peptide antibody; anti-CCP (+) was considered when >5 relative units (RU)/mL; anti-MCV: anti-mutated citrullinated vimentin antibody; anti-MCV (+) was considered when >20 U/mL. Radiographic damage was assessed using the Steinbrocker's scale.

Almost all the patients were women with a median age of 54 years, and the median disease duration at the time of the study was 12 years. Frequencies of radiographic damage according to Steinbrocker's classification revealed that 22 patients (36.1%) had mild-moderate

damage (Steinbrocker stages I-II) and 39 patients (63.9%) suffered from severe damage (Steinbrocker stages III-IV). In addition, 45.9% of patients were positive for CRP, 39.3% were positive for RF, 42.6% were positive for anti-CCP, and 55.7% were positive for anti-MCV. The *IL6* -174G/C polymorphism frequencies revealed that 49 patients (80.3%) had the GG genotype, 12 (19.7%) had the GC genotype, and no patients had the CC genotype. Regarding the *IL6* -572G/C polymorphism, the genotype distribution was as follows: 32 patients (52.5%) had the GG genotype, 26 patients (42.6%) had the GC genotype, and three (4.9%) had the CC genotype.

A comparison of the clinical, laboratory, and disease characteristics pertaining to the genotypes of both polymorphisms is presented in Table 2.

Table 2. Polymorphisms of the *IL6* gene: comparison of patient characteristics for the GG genotype *vs* the GC or CC genotypes of -174G/C and -572G/C.

	-174G/C		P	-572G/C		P
	GG (N = 49)	GC or CC (N = 12)		GG (N = 32)	GC or CC (N = 29)	
Age (years)	55 (34-79)	51 (35-67)	0.47	54 (34-79)	55 (35-71)	0.98
Disease duration (years)	12 (6-30)	15.5 (6-33)	0.07	15 (6-3)	11 (6-28)	0.09
DAS-28	4 (2-7.4)	4.13 (2.7-6.2)	0.65	4.1 (2-7.4)	3.9 (2.1-7.4)	0.93
HAQ-DI	0.8 (0-2.8)	0.33 (0-2.1)	0.33	0.7 (0-2.8)	0.5 (0-1.9)	0.59
CRP levels, mg/L	10.7 (0-174)	13.7 (6.2-35.3)	0.35	12.2 (3-45)	12.9 (0-174)	0.93
CRP (+), N (%)	25 (51.0)	7 (58.0)	0.20	18 (56.3)	12 (48.3)	1.00
RF levels, IU/mL	17 (0-1566)	55.8 (8.6-203)	0.53	58.9 (8.6-1566)	8.6 (0-528)	0.03
RF (+), N (%)	17 (34.7)	7 (55.8)	0.30	17 (53.1)	7 (24.1)	0.08
Anti-CCP levels, RU/mL	35.1 (0.01-268)	19.1 (0.01-192)	0.52	73.4 (0.01-268)	3 (0.01-207.2)	0.07
Anti-CCP (+), N (%)	22 (44.9)	4 (33.3)	0.79	16 (50)	10 (34.5)	0.05
Anti-MCV levels, U/mL	75.6 (1-190)	98.3 (62.5-228.5)	0.24	110 (8.5-228.5)	62.5 (1-190)	0.02
Anti-MCV (+), N (%)	28 (57.1)	6 (50)	0.20	18 (56.3)	16 (55.2)	0.43

Qualitative variables are reported as numerical format (percentages). Quantitative variables are reported as median and ranges. The Mann-Whitney U-test was used to compare between medians. DAS-28: disease activity score of 28 joints; HAQ-DI: health assessment questionnaire-disability index; CRP: C-reactive protein; CRP (+) was considered when >10 mg/L; RF: rheumatoid factor; RF (+) was considered when >12 international units (IU)/mL; anti-CCP: anti-cyclic citrullinated peptide antibody; anti-CCP (+) was considered when >5 RU/mL; anti-MCV: anti-mutated citrullinated vimentin antibody; anti-MCV (+) was considered when >20 IU/mL.

The -174G/C polymorphism of *IL6* showed a difference between patients carrying the GC/CC genotypes and those carrying the GG genotype with regard to disease duration (P = 0.07). With respect to the -572G/C polymorphism of *IL6*, patients carrying the GG genotype had significantly higher levels of RF (P = 0.03) and anti-MCV (P = 0.02), and were positive for anti-CCP (P = 0.05) with a non-significant trend towards higher levels of anti-CCP.

Table 3 presents a comparison of clinical and laboratory characteristics between the group with severe radiographic damage and the group with mild or moderate radiographic damage. Frequency of positivity for anti-CCPs was significantly higher in patients with severe radiographic damage (P = 0.04), and a non-significant trend was noted for higher anti-CCP levels (P = 0.08).

Table 4 shows the main results of our study, indicating that the -174G/C polymorphism of the *IL6* gene was associated with higher risk of severe radiographic damage in the hands (MLE_OR: 8.03, P = 0.03). Moreover, the C allele was associated with a higher risk of severe damage (MLE_OR: 6.98, P = 0.04). However, the GC and CC genotypes of the -572G/C polymorphism of the *IL6* gene were not associated with the risk of joint radiographic damage.

Table 3. Comparison of patient characteristics between the groups with mild or moderate radiographic damage *vs* the group with severe joint radiographic damage.

	Mild or moderate damage (Steinbrocker stages I-II) (N = 22)	Severe damage (Steinbrocker stages III-IV) (N = 39)	P
Age (years)	55 (42-79)	53 (34-71)	0.17
Diseases duration (years)	11 (6-30)	14 (6-33)	0.18
DAS-28	4.1 (2.2-7.4)	3.9 (2.0-7.4)	0.69
HAQ-DI	0.8 (0-2.1)	0.5 (0-2.8)	0.69
CRP levels, mg/L	10.7 (3-27.2)	13.6 (0-174)	0.34
CRP (+), N (%)	14 (63.6)	32 (82.0)	0.7
RF levels, IU/mL	11.3 (6.3-439.5)	42.6 (0-1563)	0.84
RF (+), *n* (%)	9 (40.9)	15 (38.5)	0.6
Anti-CCP, levels RU/mL	2.1 (0.01-181.6)	76.2 (0.01-268)	0.08
Anti-CCP (+), N (%)	5 (22.7)	21 (53.8)	0.04
Anti-MCV levels, U/mL	101.9 (7-169.4)	68.96 (1-228.5)	0.81
Anti-MCV (+), N (%)	10 (45.5)	24 (61.5)	0.7

The Mann-Whitney U-test was used to compare medians between groups. Mild or moderate joint radiographic damage: Steinbrocker stages I-II; severe radiographic damage: Steinbrocker stages III-IV. anti-CCP: anti-cyclic citrullinated peptide antibody; anti-CCP (+) was considered when ≥5 RU/mL; anti-MCV: anti-mutated citrullinated vimentin antibody. Positive anti-MCV was considered when ≥20 IU/mL.

Table 4. Risk of the -174G/C and -572G/C *IL6* gene polymorphisms and severe radiographic damage in hands.

		Steinbrocker stage (radiographic damage)		OR	95%CI	P
		Mild/moderate damage (I-II) [N = 22 (%)]	Severe damage (III-IV) [N =39 (%)]			
Genotype	-174G/C					
	G/G	21 (95.45)	28 (71.79)	1		
	G/C	1 (4.55)	11 (28.21)	8.03	1.22-187.06	0.03
Allele		2*n* = 44	2*n* = 78			
	G	43 (97.72)	67 (85.90)	1		
	C	1(2.28)	11 (14.8)	6.98	1.13-156.64	0.04
Genotype	-572G/C					
	G/G	13 (59.09)	19 (48.72)	1		
	G/C or C/C	9 (40.91)	20 (50.28)	1.5	0.52-4.50	0.44
Allele		2*n* = 44	2*n* =78			
	G	35 (79.54)	55 (70.51)	1		
	C	9 (20.46)	23(29.49)	1.62	0.68-4.07	0.28

MLE_OR: maximum likelihood odds ratio; 95%CI: 95% confidence interval. For genotype, ORs were computed using GC + CC as a risk factor and GG as the reference; 2*n*: allele numbers.

DISCUSSION

The results of the present study reveal a significant association between the GC genotype and the C allele of the -174G/C polymorphism in *IL6*, and severe joint radiographic damage in the hands of patients with a long RA disease duration. However, the G/C genotype and the C allele of the -572G/C polymorphism in *IL6* was not associated with the risk of severe joint radiographic damage in the hands.

These results contrast with the observations of Ceccarelli et al. (2011), who reported that patients with the -174G allele showed a non-significant trend towards higher rates of progression of erosive damage. In contrast, we observed that the C allele is associated with higher radiographic damage. These differences may be attributed to several factors, including the fact that Ceccarelli et al. (2011) used a musculoskeletal ultrasound score, whereas we used radiographs. Although ultrasound is more sensitive for the detection of bone erosion, it

is unable to identify bone ankylosis, which was the main outcome variable in our study. We therefore used radiographs to classify more severe radiographic damage. Second, Ceccarelli et al. (2011) noted a high variability in disease duration that was statistically significant for patients with the GG *vs* CC genotype. Disease duration constitutes a confounding factor that was not controlled in their study, whereas our sample comprised solely patients with long disease duration. However, in their examination of a study performed in Poland, Pawlik et al. (2005) identified that the -174GG genotype was associated with higher disease activity using the DAS-28. They also revealed an allelic association between the *IL6* -174G variant and increasing CRP. Pawlik et al. (2005) did not evaluate the radiographic joint damage using the Steinbrocker scale; however, the authors concluded that the *IL6* promoter polymorphism could be a factor in RA activity. More recently, Marinou et al. (2007) reported an association between the *IL6* -174G allele and increasing radiographic damage ($P < 0.005$) in patients with RF. However, in the present study, we evaluated the severity of structural radiographic damage in patients with long-term evolution of RA.

We also identified the presence of certain antibodies to citrullinated peptide antigens (ACPAs), and anti-CCPs in particular were associated with severe radiographic damage. Interestingly, the -572G/G polymorphism was associated with the presence of positivity for anti-CCPs, but not with joint damage severity according to the Steinbrocker's scale. The authors of several studies have reported an association between the presence of anti-CCPs and higher radiological damage (Marinou et al., 2007; Jilani and Mackworth-Young, 2015). A meta-analysis conducted by Jilani and Mackworth-Young (2015) clearly identifies that ACPAs (anti-CCP and anti-MCV) are associated with the severity of the joint damage. These data support our results. Currently, there are different variables associated with articular damage, including a genetic predisposition to severe damage of the joints. Therefore, we consider that the relationship we noted between the GC genotype and the C allele of the -174G/C polymorphism in *IL6* and the severity of radiographic damage is relevant; this polymorphism should be considered for inclusion in the group of potential genetic factors implicated in the development of the most extensive structural joint damage in RA. It has been noted that the presence of the C variant at position -174G/C of the *IL6* gene promoter region leads to increased transcriptional activity and thus to higher cytokine levels in serum and synovial tissues of patients with RA (Wood et al., 1992; Jones et al., 2001; Panoulas et al., 2009). Fishman et al. (1998) observed that the C allele in the -174G/C polymorphism of *IL6* suppresses transcription in HeLa cells after stimulation with lipopolysaccharide or IL-1, and GG homozygotes had circulating IL-6 concentrations approximately twice as high as those who were homozygous for the C allele.

A study assessing the frequency of the -174G/C polymorphism in the *IL6* gene showed that the -174G/G genotype of *IL6* was more prevalent in RA compared with the CC and GC genotypes (Zavaleta-Muñiz et al., 2013). Our results are in accordance with the findings described by Gao et al. (2015), who conducted a meta-analysis comparing the frequency of the *IL6* gene -174G/C polymorphism in different populations with sepsis and healthy controls, where was reported that GG was the most frequent genotype in the control group.

The authors of some cohort studies have observed that Hispanics with juvenile idiopathic arthritis may have a worse long-term functional disease prognosis compared with populations of other origins (Pelajo et al., 2013). This observation highlights the relevance of the evaluation of these polymorphisms in Mexican Mestizos and in patients of other ethnic origins.

However, we observed that the -572G/C polymorphism was not significantly associated with joint destruction. This finding is relevant because, at present, there is no

empirical evidence supporting or refuting the relationship between this polymorphism and joint damage. Therefore, to the best of our knowledge, this is the first report showing a non-association between this polymorphism and radiographic damage in hands.

IL-6 has a wide range of pleiotropic activities, including the induction of acute-phase proteins and the stimulation of T- and B-cells, which in turn stimulate synovial cells and osteoclasts, resulting in bone and cartilage damage (Kishimoto, 2005). High IL-6 levels are observed in the serum and synovial tissue of patients with RA (Tsuchida et al., 2012). It has been observed that polymorphisms in the *IL6* gene promoter region may be responsible for changes in the expression of IL-6 (Spoto et al., 2015), leading to an increase in the inflammatory process and the development of radiographic joint damage in hands. Although multifactorial mechanisms are involved in joint damage, these and other genetic variants may at least partially explain these mechanisms. In this preliminary study, we considered that *IL6* -174G/C variants should be evaluated as possible risk factors for the development of joint damage and the most adverse prognosis. Nevertheless, further studies are required to determine whether the most severe forms of joint damage are associated with these genetic variants.

A unique factor of this study is that we only selected patients with ≥5 years of disease duration, whereas patients with different disease durations formed the samples examined in most extant studies. Disease duration is a well-recognized confounder of structural damage. Hence, inclusion of patients with long disease duration is required for an assessment of severe structural damage.

Our exploratory study had several limitations, one of which was the small sample size. Nevertheless, our results showed a significant difference in the *IL6* -174G/C polymorphism in relation to the severity of structural damage. Therefore, further studies with larger samples are required to corroborate our findings. Studies that focus on populations of different ethnic origin are also needed. Another limitation of our study stemmed from the absence of a control group drawn from the normal population; this would have allowed us to compute the normal levels of autoantibodies such as RF, anti-CCP, or anti-MCV. However, we have previously reported the reference values in our patients (Díaz-Toscano et al., 2014).

In conclusion, to the best of our knowledge, this is the first study performed in Mexican Mestizo patients with RA that evaluates the association between the -174G/C and -572G/C polymorphisms of the *IL6* gene and joint damage. Our results are encouraging because they establish a potential role for the -174G/C polymorphism of *IL6* in structural damage observed in patients with RA and long disease duration for future studies. These findings require confirmation by long-term longitudinal studies evaluating the utility of the polymorphism at the time of RA diagnosis to determine the relevance of this polymorphism to the worst structural prognoses.

Conflicts of interest

The authors declare no conflict of interest.

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